



IN THE U.S. PATENT AND TRADEMARK OFFICE

Applicants: Eijiro WATANABE et al.

Serial No.: 08/992,914

Group: 1638

Filed: December 18, 1997

Examiner: D.H.Kruse

For: RAFFINOSE SYNTHASE GENES AND THEIR USE

DECLARATION UNDER 37 CFR 1.132

Honorable Commissioner of Patents and Trademarks
Washington, D.C. 20231

Sir:

I, Akitsu NAGASAWA, citizen of Japan and residing in Kamokogahara 3-28-56, Higashi-Nada-ku, Kobe-shi, Hyogo-ken, Japan, declare and say that:

1. I completed the master's course, with a major in agricultural biology, of the graduate school of Kyoto University and obtained a master's degree in agriculture at Kyoto University in March, 1984.

2. From April, 1984 to the present, I have been an employee of Sumitomo Chemical Company, Limited, the assignee of the above-identified application.

3. From April, 1984 to the present, I have been engaged in research works for plant engineering using recombination and other gene manipulation, such as cloning of plant genes, preparation and evaluation of transgenic plants.

4. I am one of the members of the research project related to the above-identified application and am familiar with the subject matter thereof.

5. I have read the Office Action mailed March 11, 2005 and the reference cited, and am familiar with the subject matter thereof.

6. To demonstrate successful identification of raffinose synthase genes in plant, I have made the following computer analysis.

ANALYSIS

1) The overall sequence homologies (%) among the amino acid sequences of raffinose synthases (RFSs), seed imbibition protein (SIP) and stachyose synthases (STSs) shown in Table 1 attached hereto were calculated based on a global multiple alignment (the alignment of sequences over their entire length) using the gene analysis software GENETYX-SV/RC for Windows version 6.1.0 (GENETYX Corporation; <http://www.sdc.co.jp/genetyx/>) with default parameters. The global multiple alignment was generated using CLUSTAL sequence analysis program. The amino acid sequences of the RFSs, SIP and STSs used to produce the global multiple alignment are as follows:

Sc-02:

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MAPPSITKTATLQDVISTIDIGNNSPLFSITLDQSRDFLANGHPFLTQV
PPNITTTTTTASSFLNLKSNKDTIPNNNTMLLQGGCFVGFNSTEPKSH
HVVPLGKLKGIKFMSIFRFKVVWTTTHWVGTVNGQELQHETQMLILDKNDSL
GRPYVLLLPILENTFRTSLQPGLNDHIGMSVESGSTHVTGSSFKACLYIH
LSNDPYSILKEAVKVIQTQLGTFKLEEKTA PSI IDKFGWCTWDAFYLVK
HPKGVWEGVKSLTDGGCPPGFVIIDDGWQSI CHDDDEDDSGMNRTSAGE
QMPCLVKYEENSKFREYENPENGKKGLGGFVRDLKEEFGSVESVYVWH
ALCGYWGGRPGVHGMFKARVVVPKVSQGLKMTMEDLAVDKIVENGVLV
PPDFAHEMF DGLHSHLESAGIDGVKVDVIHLLELLSEYGGRVELARAYY
KALTSSVKKHFKGNGV IASMEHCNDFLLGTEA ISLGRVGDDFWCSDPSG
DPNGTYWLQGGCHMVHCAYNSLWMCNFIQPDWDMFQSTHPCA EFHAASRAI
SGGPIYVSDCVGNHNFKLLKSLVLPDGSILRCQHYALPTRDCLFEDPLHN
GKTMLKIWNLNKYTGVLGLFNCQGGGWCPEARRNKSVSEFSRAVTCYASP
EDIEWCNGKTPMSTKGVDFFAVYFFKEKKLRMLKCSDR LKVSLEPFSFEL
MTVSPVKVFSKRFIQFAPIGLVNMLNSGGAIQSLEFDDNASLVKIGVRGC
GEMSVFASEKPVCKIDGVKVKFLYEDKMARVQILWPSSSTLSLVQFLF
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Sc-03:

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MAPSFSKENSKTCDEVANHDDCNTCPIISLEESNFMVNGHVILSQVPSNI
TAISKMGFDGLFVGFDAPFKARHVSVGQLKGIPFMSIFRFKVVWTTTHW
TGSNGRDLEHETQILILDKSDEGLGRPYIVILPLIEGPFRASLQPGSVDD
YVDICVESGSTKVVGDSFRAVLYIRAGDPDFKLIKDTMKEVQAHLGTFKL
LDDKTPPGIVDKFGWCTWDAFYLVKEXYGVWEGVKGLVENGVPGLVLID
DGWQSI CHDDDPITDQEGINRTSAGEQMPCLIKYEENFKFRDYKSPNIM
GHEDHPNMGMRAFVRDLKEEFKTVEHVYVWHFTGYWGGVRPNVPGLXEA
QVVT PKLSPGLEMTMEDLAVDKIVNNGIGLVQPDKAQELYEGHSHLENC
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GIDGVKVDVIHLLEMAEDYGGRVELAKTYKAITESVRKHKFGNGV IAS
MEQCNDFMLLGTTETICLGRVGDDFWPTDPSGDI NGTYWLQGCHMVHCAYN
SLWMGNFIHPDWDMFQSTHPCA EFHAASRAISGGPIYVSDVVGKHNIPLL
KRLVLADGSILRCEYHALPTKDCLFVDPLHDGKTM LKIWNLNKYNGVLGV
FNCQGGGWSRESRKNLCFSEYSKPI SCKTSPKDV EWENGHKPFP IKGVEC
FAMYFTKEKKLILSQLSDTIEISLDPFDYELIVVSPMTILPWESIAFAPI
GLVNMLNAGGAVKSLDISEDNEDKMVQVG IKGAGEMMVYSSEKPKACRVN
GEDMEFEYEESMIKVQVTWNHNSGGFTTVEYLF

Sc-04 (truncated):

MAPSISKTELNSFGLVNGNLPLSITLEGSNFLANGHPFLTEVPENIIVT
PSPIDAKSSKNNEDDDVVGCFVGFHADEPRSRHVASLGKLRGIKFMSIFR
FKVWWTTHWVGSNGHELEHETQMMLLDKNDQLGRPFVLILPILQASFRAS
LQPLDDYVDVCMESGSTRVCGSSFGSCLYVHVGHDPYQLREATKVVRM
HLGTFKLL E EKTAPVIIDKFGWCTWDAFYLVKHPSGVWEGVKGLVEGGCP
PGMVLIDDGWQAI CHDEDPITDQEGMKRTSAGEQMP CRLVKLEENYKFRQ
YCSGKDSEKGMGAFVRDLKEQFRSVEQVYVWHALCGYWGGVRPKVP GMPQ
AKVVT PKLSNGLKLTMKDLAVDKIVSNGVGLVPPHLAHLLEYGLHSRLES
AGIDGVKVDVIHLEMLSEEYGGRVELAKAYYKALTASVKKHKFGNGVIA
SMEHCNDFLLGTEAIALGRVGDDFWCTDPSGDPNGTYWLQGCHMVHCAY
NSLWMGNFIQPDWDMFQSTHPCA EFHAPLGPSLVDQFTLVIVLESTTSSC
SRASLCLMGRFCVVNTMHSPHETVCLKTPCMMGRQCSKFGISTNIQVFWV
YLIAKEVGGVP

Sc-05:

MAPPSVIKSDAAVNGIDLSGKPLFRLEGSDLLANGHVVLTDVPVNVTVTA
SPYLADKDGEPVDASAGSFIGFNLDGEPRSRHVASIGKL RDIRFMSIFRF
KVWWTTHWVGSKGSDIENETQIIILENSGSGRPYVLLLPLLEG SFRSSFQ
PGEDDDVAVCVESGSTQVTGSEFRQVYVYHAGDDPFLVKDAMKVVRVHM
NTFKLLEEKXPPGIVDKFGWCTWDAFYLTVNPDGVHKGVKCLVDGGCPPG
LVLIDDGWQSIGHDSIDVEGMSCTVAGEQMP CRLKFQENFKFRDYVS
PKDKNEVGMAFVRDLKEEFSTVDYIYVWHALCGYWGLRPGAPTLPPST
IVRPELSPGLKLTMQDLAVDKIVDTGIGFVSPD MANEFYGLHSHLQNVG
IDGVKVDVIHILEMLCEKYGGRVDLAKAYFKALTSSVNKHFDGNGV IASM
EHCNDFMFLGTEAISLGRVGDDFWCTDPSGDI NGTYWLQGCHMVHCAYNS
LWMGNFIQPDWDMFQSTHPCA EFHAASRAISGGPIYISDCVGHDFDLLK
RLVLPDGSILRCEHYALPTRDRLFEDPLHDGKTM LKIWNLNKYTG IIGAF
NCQGGGWCRETRRNQCFSQCVNTLTATTNPKDVEWNSGNNPISVENVEEF
ALFLSQSKKLVLSGPND DLEITLEPFFKELITVSPVVTIEGSSVQFAPIG
LVNMLNTSGAIRSLVYHEESVEIGVRGAGEFRVYASRK PASCKIDGEVVE
FGYEESMVMVQVPWSAPEG LSSIKYEF

PsRFS:

MAPPSITKTATQQDVI STVDIGNSPLLSISLDQSRNFLVNGHPFLTQVPP
NITTTTTSTPSPFLDFKSNKDTIANNNNTLQQQGC FVGNTTEAKSHHV
PLGKLKGIKFTSIFRFKVVWTHHWGTNGHELQHETQILILDKNISLGRP
YVLLLP ILENSFRTSLQPLNDYVDMSVESGSTHVTGSTFKACLYLHLSN
DPYRLVKEAVKVIQTKLGTFTLEEKTPPSIEKFGWCTWDAFYLVKHPK
GVWEGVKALTDGGCPPGFVIIDDGWQSI SHDDDDPVTERDGMNRTSAGEQ
MPCRLIKYEENYKFREYENGDNNGGKGLVGFVRDLKEEFRSVESVYVWHA
LCGYWGGVRPKVCGMPEAKVVVPKLSPGVKMTMEDLAVDKIVENGVLVP
PNLAQEMFDGIHSHLESAGIDGVKVDVIHLLLELSEEYGGRVELAKAYYK
ALTSSVNHFKGNGV IASMEHCNDFLLGTEAISLGRVGDDFWCCDP
SGDPNGTYWLQGGCHMVHCAYNSLWMGNFIHPDWDMFQSTHPCAEFHAASRAIS
GGPVYVSDCVGNHNFKLLKSFVLPDGSILRCQHYALPTRDCLFEDPLHNG
KTMLKIWNLNKYAGVLGLFNCQGGGWCPETRRNKSASEFSHAVTCYASPE
DIEWCNGKTPMDIKGVDVFAVYFFKEKKLSMKCSDRLEVSLEPFSFELM
TVSPLKVFSKRLIQFAPIGLVNMLNSGGAVQSLEFDDASLVKIGVRGCG
ELSVFASEKPVCKIDGVSVEFDYEDKMVRVQILWPGSSTLSLVEFLF

Aj-05:

MAPSFKNGGSNVVSFDGLNDMSSPFAIDGSDFTVNGHSFLSDVPENIVAS
PSPYTSIDKSPVSVGCFVGFDASEPDSRHVVSIGKLDIRFMSIFRFKVV
WTHHWGRNGGDLESETQIVILEKSDSGRPYVLLPIVEGPFRTSIQPGD
DDFVDVCVESGSSKVVDASFRSMLYLHAGDDPFALVKEAMKIVRTHLGT
FRLLEEKTPPGIVDKFGWCTWDAFYLT VHPQGVIEGVRHLVDGGCPPGLVL
IDDGWQSIGHSDPI TKEGMNQT VAGEQMPCRLLKFQENYKFRDYVNP
KATGPRAGQKGMKAFIDELKGEFKTVEHVYVWHALCGYWGGLRPQVPGLPEA
RVIQPVLSPLQMTMEDLAVDKIVLHKVGLVPPEKAEEMYEGLHAHLEKV
GIDGVKIDVIHLLLEMLCEDYGGRVDLAKAYYKAMTKSINKHFKGNGV
IASMEHCNDFMFLGTEAISLGRVGDDFWCTDP SGDPNGTFWLQGGCHMVHC
ANDSLWMGNFIHPDWDMFQSTHPCAAFHAASRAISGGPIYVSDSVGKHNF
DLLKKLVLPDGSILRSEYYALPTRDCLFEDPLHNGETMLKIWNLNKFTGVI
GAFNCQGGGWCRETRRNQCFSQYSKRVT SKTNPKDIEWHSGENPISIEGV
KTFALYLYQAKKILSKPSQDLDIALDPFEFELITVSPVTKLIQTSLHFAP
IGLVNMLNTSGAIQSVDYDDDLSSVEIGVKGC GEMRVFASKKPRACRIDGE
DVGFKYDQDQMVVVQVPWPIDSSSGGISVIEYLF

HvSIP:

MTVTPQITVGDRLAVRGRTVLSGVPDNVTAAHAAGAGLVDGAFVGATAA
EAKSHHVFTFGTLRDCRFMCLFRFKLWWM TQRMGTSGRDVPLETQFILIE

VPAAAGNDDGDSSDGDSEPVYLVMLPLLEGQFRTVLQGNDQDELQICIES
 GDKAVETEQQGMNNVYVHAGTNPFDITQAVKAVEKHTQTFHHREKKTVP
 FVDWFGWCTWDAFYTDVTADGVKQGLRSLAEGGAPPRFLIIDDGWQQIGS
 ENKDDPGVAVQEGAQFASRLTGIRENTKFQSEHNQEETPGLKRLVDETKK
 EHGVSXVYVWHAMAGYWGCVKPSAAGMEHYEPALAYPVQSPGVTGNQPD
 VMDSLSVLGLGLVHPRRVHRFYDELHAYLAACGVDGVKVDVQNI VETLGA
 GHGGRVALTRAYHRALEASVARNFDPNGCISCMCHNTDMLYSAKQTAVVR
 ASDDFYPRDPASHTVHISSVAYNTLFLGEFMQPDWDMFSLHPAAEYHGA
 ARAIGGCP IYVSDKPGNHNFDLLRKLVLPGDSVLRAQLPGRPTRDCLFSD
 PARDGASLLKIWNMNCAGVGVFNCQGAGWCRVAKKTRIHDEAPGTLTG
 SVRAEDVEAIAQAAGTDWGGEAVVYAHRADELVRLPRGATLPVTLKRLE
 YELFHVCPVRAVAPGVSFAPIGLLHMFNAGGAVEECTVETGEDGNAVVG
 RVRGCGRFGAYCSRRPAKCSVDSADVEFTYDSDTGLVTADVPVPEKEMYR
 CALEIRV

AmSTS:

MAPPYDPIPIPIPM SAILNFLSSTVKDNSFELLDGTL SVKNVPI LTDIPS
 NVSFSSFSIIVQSSEAPVPLFQRAQSLSSSGGFLGFSQNEPSSRLMNSLG
 KFTDRDFVSI FRFKTWWSTQWVGTTGSDIQMETQWIMLDVPEIKSYAVVV
 PIVEGKFRSALFPKGKGHILIGAESGSTKVKTSNFDIAIYVHVSENPYTL
 MRDAYTAVRVHLNTFKLIEEKSAPPLVNKFGWWTWDAFYLTVEPAGIYHG
 VQEFADGGLTPRFLIIDDGWQSINDDNDPNEDAKNLVLGGTQMTARLHR
 LDECEKFRKYKGGMSGPNRPPFDPKPKLLISKAIEIEVAEKARDKAAQ
 SGVTDLARYEAEIEKLTKELDQMFGGGGEETSSGKSCSSCSCKSDNFGMK
 AFTKDLRTNFKGLDDIYVWHALAGAWGGVRPGATHLNAKIVPTNLSPGLD
 GTMTDLAVVKIIEGSTGLVDPDQAEDFYDSMHSYLSVVGITGVKVDVIHT
 LEYISEDYGGRVELAKAYYKGLSKSLAKNFNGTGLISSMQQCNDFFLLGT
 EQISMGRVGGDFWFQDPNGDPMGVYWLQGVHMIHCAYNSMWMGQFIQPDW
 DMFQSDHPGGYFHAGSRAICGGPVYVSDSLGGHNFDLLKKLVFNDGTIPK
 CIHFALPTRDCLFKNPLFDSKITLKIWNFNKYGGVIGAFNCQGAGWDPKE
 QRIKGYSCYKPLSGSVHVSIGIEFDQKKEASEMGEAEYAVYLSEAELLS
 LATRDS DPIKITIQSSTFEIFSFPVKKLGEGVKFAPIGLTNLFNAGGTI
 QGLVYNEGIAKIEVKGDGFLAYSSVVPKKAYVNGAEKVFAWSGNGKLEL
 DITWYEECGGISNVTFVY

PsSTS-1:

MAPPLNSTTSNLIKTESIFDLSEKFKVKGFPLFHDVPENVSFERSFSSIC
 KPSESNA PPSLLQKVLAYSHKGGFFGF SHETPSDRLMNSIGSFNGKDFLS
 IFRFKTWWSTQWIGKSGSDLQMETQWILIEVPETKSYVVIPIIEKCFRS
 ALFPGFNDHVKIIAESGSTKVKESTFNSIAYVHFSENPYDLMKEAYS AIR
 VHLNSFRLL EKTIPNLVDKFGWCTWDAFYLT VNPIGIFHGLDDFSKGGV

EPRFVI IDDGWQSI SFDGYDPNEDAKNLVLGGEQMSGRLHRFDECYKFRK
YESGLLLGPNSPPYDPNNFTDLILKGLIEHEKLRKKREEAISSKSSDLAEI
ESKIKKVVKEIDDLFGGEQFSSGEKSEMKEYGLKAF TKDLRTKFKGLDD
VYVWHALCGAWGGVRPETTHLDTKIVPCKLSPGLDGTMEDLAVVEISKAS
LGLVHPSQANELYDSMHSYLAESGITGVKVDVIHSLEYVCDEYGGRVDLA
KVYYEGLTKSIVKNFNGNGMIASMQHCNDDFFLGTKQISMGRVGDDFWFQ
DPNGDPMGSFWLQGVHMIHCSYNSLWMGQMIQPDWDMFQSDHVC AKFHAG
SRAICGGPIYVSDNVGSHDFDLIKKLVPDGTIPKCIYFPLPTRDCLFKN
PLFDHTTVLKIWNFNKYGGVIGAFNCQGAGWDPIMQKFRGFPECYKPIPG
TVHVTEVEWDQKEETSHLGKAEYVVYLNQAEELSLMTLKSEPIQFTIQP
STFELYSFVPVTKLCGGIKFAPIGLTNMFNSGGTVIDLEYVGNGAKIKVK
GGGSFLAYSSSEPKKFQLNGCEVDFEWLGDGKLCVNVPWIEEACGVSDME
IFF

PsSTS-2:

MAPPLNSTTSNLIKTESIFDLSEKFKVKGFPLFHDVPENVSFERSFSSIC
KPSESNA PPSLLQKVLAYSHKGGFFGFSHETPSDRLMNSLGSFNGKDFLS
IFRFTWWSTQWIGKSGSDLQMETQWILIEVPETKSYVVIPIIEKCFRS
ALFPGFNDHVKIIAESGSTKVKESTFNSIAYVHFSENOPYDLMKEAYIAIR
VHLNSFRLL EKTIPNLVDKFGWCTWDAFYLT VNPIGIFHGLDDFSKGGV
EPRFVI IDDGWQSI SFDGCDPNEDAKNLVLGGEQMSGRLHRFDECYKFRK
YESGLLLGPNSPPYDPKKFTDLILKGLIEHEKLRKKREEAISSKSSDLAEI
ESKIKKVVKEIDDLFGGEQFSSVEKSEMKEYGLKAF TKDLRTKFKGLDD
VYVWHALCGAWGGVRPETTHLDTKFVPCKLSPGLDGTMEDLAVVEISKAS
LGLVHPSQANELYDSMHSYLAESGITGVKVDVIHSLEYVCDEYGGRVDLA
KVYYEGLTKSIVKNFNGNGMIASMQQCNDFFLGTKQISMGRVGDDFWFQ
DPNGDPMGSFWLQGVHMIHCSYNSLWMGQMIQPDWDMFKSDHVC AKFHAG
SRAICGGPIYVSDNVGSHDFDLIKKLVPDGTIPKCIYFPLPTRDCLFKN
PLFDHTTLLKIWNFNKYGGVIGAFNCQGAGWDPIMQKFRGFPECYKPIPG
TVHVTQVEWDQKEETSHFGKAEYVVYLNQAEELCLMTLKSEPIQFTIQP
STFELYSFVPVTKLCGGIKFAPIGLTNMFNSGGTVIDLEYVGNGAKIKVK
GGGSFLAYSSSEPKKFQLNGCEVDFEWLGDGKLCVNVPWIEEACGV S

SaSTS:

MAPPNDPISSIFSPLISVKKDNAFELVGGKLSVKNVPLLSEIPSNVTFKS
FSSICQSSGAPAPLYNRAQSLSNCGGFLGFSQKESADSVTNSLGKFTNRE
FVSI FRFTWWSTQWVGTS GSDIQMETQWIMNLPEIKSYAVVIPIVEGK
FRSALFPGKDGHVLSAESGSTCVKTTSTFTSIAYVHVSDNPYTLMKDGYT
AVRVHLDTFKLIIEKSAPPLVNKFGWCTWDAFYLTVEPAGI WNGVKEFS
GGFSRFLIIDDGWQSIINIDGQDPNEDAKNLVLGGTQMTARLHRFDECEK
FRKYKGGSMMPKVPYFDPKKPKLLISKAIEIEGVEKARDKAIQSGITDL

SQYEIKLKKLNKELDEMFGGGGNDEKGSSKGCSDCCKSQNSGMKAFTND
 LRTNFKGLDDIYVWHALAGAWGGVKPGATHLNAKIEPCKLSPGLDGTMTD
 LAVVKILEGSI GLVHPDQAEDFYDSMHSYLSKVGITGVKVDVIHTLEYVS
 ENYGG RVELGKAYYKGLSKSLKKNFNGSGLISSMQQCNDFFLLGTEQISM
 GRVGDDFWFQDPNGDPMGVFWLQGVHMIHCAYNSMWMGQIHPDWDMFQS
 DHCSAKFHAGSRAICGGPVYVSDSLGGHDFDLLKKLVFNDGTIPKCIHFA
 LPTRDCLFKNPLFDSKITLKIWNFNKYGGVVGAFNCQGAGWDPKEQRIKG
 YSECYKPLSGSVHVSDIEWDQKVEATKMGEAEEYAVYLTESEKLLLTTP
 SDPIPF TLKSTTFEIFSFPV IKKLGGQVKFAP IGLTNLFNSGGTIQGVVY
 DEGVAKIEVKG DGKFLAYSSSVPKRSYLN GEEVEYKWSGNGKVEVDVPWY
 EECGGISNITFVF

VaSTS:

MAPPNDPVNATLGLEPSEKVF DLSDGKLT VKGVL LSHVPENVT FSSSFSS
 ICVPRDAPSSILQRVTAASHKGGFLGF SHVSPSDRL INSLGSFRGRNFLS
 IFRKTTWWSTQWVGNSGSDLQMETQWILIEVPETESYVVIPIIEKSFRS
 ALHPGSDDHVKI CAESGSTQVRASSFGAIAYVHVAETPYNLMREAYSALR
 VHLDSFRLL EKTVPRI VDKFGWCTWDAFYLT VNPVGVWHGLKDFSEGGV
 APRFVVIDDGWQSVNFDDDPNEDAKNLVLGGEQMTARLHRFEEDKFRK
 YQKGLLLGPNAPSFNPETIKELISKGIEAEHLGKQAAAI SAGGSDLAEIE
 LMIVKVREEIDDLFGGKGKESNESGGCCCKAAECGGMKDFTTDLRTEFKG
 LDDVYVWHALCGGWGVRPGTTHLDSKIIPCKLSPGLVGTMKDLAVDKIV
 EGSIGLVHPHQANDLYDSMHSYLAQTGVTGVKIDVIHSLEYVCEEYGGRV
 EIAKAYYDGLTNSI IKNFNGSGI IASMQQCNDFFFLGTKQIPFGRVGDDF
 WFQDPNGDPMGVFWLQGVHMIHCSYNSLWMGQIIPDWDMFQSDHECAKF
 HAGSRAICGGPVYVSDSVGSHDFDLIKKLVFPDGTVPKCIYFPLPTRDCL
 FRNPLFDQKTVLKIWNFNKYGGVIGAFNCQGAGWDPKGKKFKGFPECYKA
 ISCTVHVTEVEWDQKKEAEHMGKAEYVYVLNQA EVLHLMTPVSEPLQLT
 IQPSTFELYNFVPVEKLGSSNIKFAP IGLTNMFNSGGTIQELEYIEKDVK
 VKVKGGRFLAYSTQSPKKFQLNGSDAAFQWLPDGKLTNLAWIEENDGV
 SDLAIFF

The calculated overall sequence homologies (%) are shown in Table 2 attached hereto. The homologies between RFSs and SIP are less than 40%. The homologies between RFSs and STSs are not higher than 45%. On the other hand, the homologies among RFSs are all 50% or higher. Thus, the homologies among RFSs are higher than those homologies between RFSs and SIP and between RFSs and STSs.

A molecular phylogenic tree of the RFSs, SIP and STSs shown in Table 1 is

drawn in Figure 1 attached hereto. The molecular phylogenetic tree is drawn by the UPGMA method using the gene analysis software GENETYX-SV/RC for Windows version 6.1.0 (GENETYX Corporation; <http://www.sdc.co.jp/genetyx/>) with default parameters. In the molecular phylogenetic tree, RFSs, SIP and STSs form different groups respectively.

In summary, Table 2 and Figure 1 show that RFSs, SIP and STSs can be distinguished from one another based upon a comparison of their amino acid sequences.

2) Attached Table 3 shows the identities obtained using the BLAST program for the amino acid sequences of RFSs, SIP and STSs shown in Table 1. Among Sc-02, Sc-03, Sc-04 and Sc-05, the identities were obtained by searching the "patent database" provided by NCBI (National Center for Biotechnology Information) with default parameters, using the amino acid sequence of each protein as the "query", and using "Protein query vs. translated database (tblastn)" of the NCBI BLAST program. Also, other identities were obtained by searching the "non-redundant database" provided by NCBI with default parameters, using the amino acid sequence of each protein as the "query", and using "Protein-protein BLAST (blastp)" of the NCBI BLAST program. The above-identified amino acid sequences of the RFSs, SIP and STSs are used as the "query" except that the amino acid sequence of Sc-04 used as the "query" is as follows:

Sc-04 (full-length):

```
MAPSISKTVELNSFGLVNGNLPLSITLEGSNFLANGHPFLTEVPENIIVT  
PSPIDAKSSKNNEDDDVVGCFVGFHADEPRSRHVASLGKLRGIKFMSIFR  
FKVWWTTHWVGSNGHELEHETQMMLLDKNDQLGRPFVLIPLILQASFRAS  
LQPLDDYVDVCMESGSTRVCGSSFGSCLYVHVGHPYQLLREATKVVRM  
HLGTFKLLEEKTA PVIIDKFGWCTWDAFYLVHPSGVWEGVKGLVEGGCP  
PGMVLIDDGWQAI CHDEDPITDQEGMKRTSAGEQMPCLVKLEENYKFRQ  
YCSGKDSEKGMGAFVRDLKEQFRSVEQVYVWHALCGYWGGVRPKVPGMPQ  
AKVVTPKLSNGLKLTMKDLAVDKIVSNGVGLVPPHLAHLLEYGLHSRLES  
AGIDGVKVDVIHLLLEMLSEEYGGRVELAKAYYKALTASVKKHFKGNGVIA  
SMEHCNDFLLGTEAIALGRVGDDFWCTDPSGDPNGTYWLQGCHMVHCAY  
NSLWMGNFIQPDWDMFQSTHPCA EFHAASRAISGGPVYVSDCVGKHNFKL  
LKSLALPDGTILRCQHYALPTRDCLFEDPLHDGKTM LKIWNLNKYTGVLG
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LFNCQGGGWCPVTRRNKSASEFSQTVTCLASPQDIEWSNGKSPICIKGMN
VFAVYLFKDHKLKLMKASEKLEVSLEPFTFELLTVSPVIVLSKKLIQFAP
IGLVNMLNTGGAIQSMEFDNHIDVVKIGVRGCGEMKVFASEKPVSCKLDG
VVVKFDYEDKMLRVQVPWPSASKLSMVEFLF

As shown in Table 3, the identities between RFSs and SIPs are about 40%. The identities between RFSs and STSs range from about 40% to about 50%. On the other hand the identities among RFSs are 60% or higher. The identities among STSs are also 60% or higher. That is, the identities among RFSs or the identities among STSs are higher than the identities between RFSs and SIP or the identities between RFSs and STSs. Thus, RFSs, SIP or STSs can be distinguished based on the results of analysis using BLAST program.

3) Attached Table 4 shows the identities obtained using another BLAST program for the amino acid sequences of RFSs, SIP and STSs shown in Table 1. All possible pair-wised amino acid sequence comparison were made by the "Blast 2 Sequences" program from NCBI (<http://www.ncbi.nlm.nih.gov/blast/bl2seq/bl2.html>). Sequence identities were calculated using default parameters, program; blastp, matrix; BLOSUM62, open gap penalty; 11, extension gap penalty; 1, gap x_dropoff; 50, expect; 10.0, and word size; 3. The amino acid sequences of the RFSs, SIP and STSs used to calculate sequence identities are identical to those used as the "query" to obtain identities shown in Table 3. Results were essentially the same with former two types of comparison.

4) In conclusion, raffinose synthases (RFSs), seed imbibition protein (SIP) and stachyose synthases (STSs) were clearly distinguished from one another based on comparison of their amino acid sequences.

7. I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonments, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the above-identified application or any patent issued thereon.

This 6th day of September, 2005


Akitsu NAGASAWA



Table 1

Code	Protein*	Organism	Accession**	Reference	Author/Assignee
Sc-03	RFS	<i>Beta vulgaris</i>	E37133	09/301,766	Sumitomo Chemical
Sc-05	RFS	<i>Brassica juncea</i>	E36417	09/301,766	Sumitomo Chemical
Sc-02	RFS	<i>Vicia faba</i>	E24423	08/992,914	Sumitomo Chemical
Sc-04	RFS	<i>Glycine max</i>	E24424	08/992,914	Sumitomo Chemical
Aj-05	RFS	<i>Cucumis sativus</i>	AF073744	Family GH36***	Ohsumi et al.
PsRFS	RFS	<i>Pisum sativum</i>	AJ426475	Family GH36	Peterbauer et al.
HvSIP	SIP	<i>Hordeum vulgare</i>	M77475	Family GH36	Heck et al.
PsSTS-1	STS	<i>Pisum sativum</i>	AJ311087	Family GH36	Peterbauer et al.
PsSTS-2	STS	<i>Pisum sativum</i>	AJ512932	Family GH36	Peterbauer et al.
VaSTS	STS	<i>Vigna angularis</i>	Y19024	Family GH36	Peterbauer et al.
AmSTS	STS	<i>Alonsoa meridionalis</i>	AJ487030	Family GH36	Voitsekhovskaja
SsSTS	STS	<i>Stachys affinis</i>	AJ344091	Family GH36	Pesch and Schmitz

*Protein: RFS, Raffinose synthase; SIP, Seed Imbibition Protein; STS, Stachyose synthase.

**Accession: GenBank Accession Number.

***Family GH36: glycoside hydrolase family 36 (see Carbohydrate-Active Enzymes (CAZy) database: http://afmb.cnrs-mrs.fr/CAZY/GH_36.html)

Table 2

[illegible]

Table 3

[illegible]

Table 4

[illegible]

Fig. 1

[GENETYX : Evolutionary tree]
 Date : 2004.2.4
 Method: UPGMA

